

What is claimed is:

1. An isolated nucleic acid molecule comprising a sequence of nucleotides which encodes or is complementary to a sequence which encodes a mammalian α -N-acetylglucosaminidase or fragment or derivative thereof.
- 5 2. The isolated nucleic acid molecule according to claim 1 wherein the nucleotides are deoxyribonucleotides.
3. The isolated nucleic acid molecule according to claim 2 wherein said molecule is a cDNA.
4. The isolated nucleic acid molecule according to claim 2 wherein
10 said molecule is a genomic DNA molecule.
5. The isolated nucleic acid according to claim 1 wherein the mammal is a human.
6. The isolated nucleic acid according to claim 5 wherein the α -N-acetylglucosaminidase is of liver, kidney or placenta origin.
- 15 7. The isolated nucleic acid molecule according to claim 1 having a nucleotide sequence substantially as set forth in SEQ ID NO: 1 or complementary thereto or having at least 40% similarity to all or part thereof.
8. The isolated nucleic acid molecule according to claim 1 having a nucleotide sequence substantially as set forth in SEQ ID NO: 3 or complementary
20 thereto or having at least 40% similarity to all or part thereof.
9. The isolated nucleic acid molecule according to claim 7 wherein the percentage similarity is at least 60%.
10. The isolated nucleic acid molecule according to claim 9 wherein the percentage homology is at least 80%.
- 25 11. The isolated nucleic acid molecule according to claim 1 wherein the α -N-acetylglucosaminidase or fragment or derivative thereof encoded by said

molecule comprises an amino acid sequence substantially identical to SEQ ID NO:2 or is at least .40% similar to all or a part thereof.

12. The isolated nucleic acid molecule according to claim 11 wherein the percentage similarity to SEQ ID NO: 2 is at least 60%.

5 13. The isolated nucleic acid molecule according to claim 12 wherein the percentage similarity to SEQ ID NO:2 is at least 80%.

14. The isolated nucleic acid molecule according to claim 1 wherein said molecule is carried by a vector capable of replication in a eukaryotic cell and/or a prokaryotic cell.

10 15. The isolated nucleic acid molecule according to claim 14 wherein the vector is an expression vector.

16. The isolated nucleic acid molecule according to claim 15 wherein the expression vector is capable of being expressed in cells derived from a eukaryote.

15 17. The isolated nucleic acid molecule according to claim 16 wherein the expression vector is further capable of being expressed in cells derived from a mammal.

18. The isolated nucleic acid molecule according to claim 17 wherein the expression vector is further capable of being expressed in CHO cells.

20 19. A recombinant mammalian α -N-acetylglucosaminidase or fragment or derivative thereof.

20. The recombinant mammalian α -N-acetylglucosaminidase according to claim 19 in substantially pure form.

25 21. The recombinant mammalian α -N-acetylglucosaminidase according to claim 19 when expressed in mammalian, yeast or insect cells.

22. The recombinant mammalian α -N-acetylglucosaminidase according to claim 21 when expressed in mammalian cells.

23. The recombinant mammalian α -N-acetylglucosaminidase according to claim 21, wherein the cells are capable of glycosylating said
5 recombinant mammalian α -N-acetylglucosaminidase.

24. The recombinant mammalian α -N-acetylglucosaminidase according to claim 23 wherein the cells are capable of N-glycosylating said recombinant mammalian α -N-acetylglucosaminidase.

25. The recombinant mammalian α -N-acetylglucosaminidase
10 according to claim 24 wherein the cells are CHO cells.

26. The recombinant mammalian α -N-acetylglucosaminidase according to claim 19 wherein said recombinant α -N-acetylglucosaminidase is in a glycosylated form.

27. The recombinant mammalian α -N-acetylglucosaminidase
15 according to claim 26 wherein the molecular weight of the glycosylated form as determined using SDS/PAGE is at least approximately 79 kDa.

28. The recombinant α -N-acetylglucosaminidase according to claim 26 wherein the molecular weight of the glycosylated form as determined using SDS/PAGE is at least approximately 79 kDa to 89 kDa.

20 29. The recombinant mammalian α -N-acetylglucosaminidase according to claim 19 comprising a sequence of amino acids substantially the same as a human α -N-acetylglucosaminidase.

30. The recombinant mammalian α -N-acetylglucosaminidase according to claim 19 when fused to another proteinaceous molecule.

25 31. The recombinant mammalian α -N-acetylglucosaminidase according to claim 30 wherein the other proteinaceous molecule is an enzyme, reporter molecule, purification site and/or a signal sequence.

32. The recombinant mammalian α -N-acetylglucosaminidase according to claim 19 comprising an amino acid sequence substantially as set forth in SEQ ID NO:2 or having at least 40% similarity to all or part thereof.

5 33. The recombinant mammalian α -N-acetylglucosaminidase according to claim 32 wherein the percentage similarity to SEQ ID NO:2 is at least 60%.

34. The recombinant mammalian α -N-acetylglucosaminidase according to claim 33 wherein the percentage similarity to SEQ ID NO:2 is at least 80%.

10 35. A recombinant α -N-acetylglucosaminidase produced by expression of a nucleic acid molecule which encodes or is complementary to a sequence which encodes a mammalian α -N-acetylglucosaminidase or fragment thereof and wherein the molecule is carried by a vector capable of replication in a eukaryotic or prokaryotic cell.

15 36. The recombinant α -N-acetylglucosaminidase according to claim 35 when glycosylated.

37. A method of diagnosing a mutation in a gene which encodes α -N-acetylglucosaminidase in a human patient said method comprising contacting genomic DNA or RNA derived from said patient with one or more isolated DNA
20 molecules or oligonucleotides comprising at least 10 contiguous nucleotides derived from SEQ ID NO: 1 or SEQ ID NO:3 or a complementary strand thereof for a time and under conditions sufficient for hybridisation to occur and then detecting said hybridisation using a detection means.

38. The method according to claim 37 wherein the detection means is a
25 reporter molecule covalently attached to the isolated DNA molecule or oligonucleotide.

39. The method according to claim 38 wherein the reporter molecule is ^{31}P , ^{35}S or biotin.

40. The method according to claim 37 wherein the detection means is a polymerase chain reaction format.

41. The method according to claim 40 wherein the polymerase chain reaction format is selected from the list comprising SSCP, AMD, AFLP, IRS-PCR, iPCR or RT-PCR, amongst others.

42. The method according to claim 41 wherein the polymerase chain reaction format is SSCP.

43. The method according to claim 42 wherein the isolated DNA molecule or oligonucleotide comprises at least 20 contiguous nucleotides derived from SEQ ID NO: 1 or SEQ ID NO:3 or a complementary strand thereof.

44. The method according to claim 43 wherein the isolated DNA molecule or oligonucleotide comprises at least 50 contiguous nucleotides derived from SEQ ID NO: 1 or SEQ ID NO:3 or a complementary strand thereof.

45. The method according to claim 44 wherein the isolated DNA molecule or oligonucleotide comprises at least 7 contiguous nucleotides derived from SEQ ID NO: 1 or SEQ ID NO:3 or a complementary strand thereof.

46. A method for treating a patient suffering from α -N-acetylglucosaminidase deficiency said method comprising administering to said patient an effective amount of recombinant mammalian α -N-acetylglucosaminidase or an active fragment or derivative thereof.

47. The method according to claim 46 wherein the mammalian α -N-acetylglucosaminidase comprises a sequence of amino acids substantially the same as the amino acid, sequence of human α -N-acetylglucosaminidase.

48. The method according to claim 47 wherein the patient is suffering from mucopolysaccharidosis type IIIB.

49. The method according to claim 46 wherein the recombinant α -N-acetylglucosaminidase is produced in mammalian cells.

50. The method according to claim 49 wherein the mammalian cells are capable of glycosylating the recombinant α -N-acetylglucosaminidase produced therein.

52. The method according to claim 50 wherein the recombinant α -N-acetylglucosaminidase is in a glycosylated form.

53. The method according to claim 51 wherein the glycosylated form of the recombinant α -N-acetylglucosaminidase has a molecular weight as determined using SDS/PAGE of at least approximately 79kDa.

54. The method according to claim 52 wherein the glycosylated form of the recombinant α -N-acetylglucosaminidase has a molecular weight as determined using SDS/PAGE of at least approximately 79 kDa to 89 kDa.

55. The method according to claim 46 wherein the recombinant α -N-acetylglucosaminidase comprises a sequence of amino acids substantially as set forth in SEQ ID NO:2 or having at least 40% similarity to all or a part thereof.

56. The method according to claim 54 wherein the percentage similarity to SEQ ID NO:2 is at least 60%.

57. The method according to claim 55 wherein the percentage similarity to SEQ ID NO:2 is at least 80%.

58. A method for treating a patient suffering from α -N-acetylglucosaminidase deficiency said method comprising administering to said patient an effective amount of recombinant mammalian α -N-acetylglucosaminidase or an active fragment or derivative thereof, wherein said recombinant mammalian α -N-acetylglucosaminidase is produced by expression of a nucleic acid molecule according to claim 14.

59. The method according to claim 46 wherein administration of the recombinant mammalian α -N-acetylglucosaminidase is by oral, intravenous, suppository, intraperitoneal intramuscular, intranasal, intradermal or subcutaneous

administration by infusion or implantation or by enzyme replacement therapy or by gene therapy.

59. The method according to claim 58 wherein the method of administration is by enzyme replacement therapy.

5 60. A pharmaceutical composition comprising a recombinant mammalian α -N-acetylglucosaminidase or an active fragment or derivative thereof and one or more pharmaceutically acceptable carriers and/or diluents.

61. The pharmaceutical composition according to claim 60 wherein the recombinant mammalian α -N-acetylglucosaminidase comprises a sequence of
10 amino acids substantially the same as human α -N-acetylglucosaminidase.

62. The pharmaceutical composition according to claim 60 wherein the recombinant mammalian α -N-acetylglucosaminidase is produced in a mammalian cell.

63. The pharmaceutical composition according to claim 62 wherein the
15 mammalian cell is a CHO cell line which is capable of glycosylating the recombinant mammalian α -N-acetylglucosaminidase.

64. The pharmaceutical composition according to claim 60 wherein the α -N-acetylglucosaminidase is glycosylated.

65. The pharmaceutical composition according to claim 64 wherein the
20 recombinant α -N-acetylglucosaminidase has a molecular weight as determined using SDS/PAGE of at least approximately 79kDa.

66. The pharmaceutical composition according to claim 65 wherein the recombinant α -N-acetylglucosaminidase has a molecular weight as determined using SDS/PAGE of approximately 79kDa to 89kDa.

25 67. The pharmaceutical composition according to claim 60 wherein the recombinant α -N-acetylglucosaminidase comprises a sequence of amino acids

substantially as set forth in SEQ ID NO: 2 or having at least 40% similarity to all or part thereof.

68. The pharmaceutical composition according to claim 67 wherein the percentage similarity to SEQ ID NO:2 is at least 60%.

5 69. The pharmaceutical composition according to claim 68 wherein the percentage similarity to SEQ ID NO:2 is at least 80%.

70. A pharmaceutical composition comprising recombinant mammalian α -N-acetylglucosaminidase or an active fragment or derivative thereof and one or more pharmaceutically acceptable carriers and/or diluents
10 wherein said recombinant mammalian α -N-acetylglucosaminidase is produced by expression of a nucleic acid molecule according to claim 35.

71. A pharmaceutical composition comprising recombinant mammalian α -N-acetylglucosaminidase or an active fragment or derivative thereof and one or more pharmaceutically acceptable carriers and/or diluents when
15 used in the method for treating a patient suffering from α -N-acetylglucosaminidase .

72. Use of recombinant mammalian α -N-acetylglucosaminidase or an active fragment or derivative thereof in the manufacture of a medicament for the treatment of α -N-acetylglucosaminidase deficiency in a patient.

20 73. The use according to claim 72 wherein the recombinant mammalian α -N-acetylglucosaminidase comprises a sequence of amino acids substantially the same as the amino acid sequence of human α -N-acetylglucosaminidase.

74. The use according to claim 72 wherein the patient is suffering from
25 mucopolysaccharidosis type IIIB.

75. The use according to claim 74 wherein the recombinant is α -N-acetylglucosaminidase expressed in mammalian cells.

76. The use according to claim 75 wherein the cells are CHO cells.

77. The use according to claim 72 wherein the α -N-acetylglucosaminidase is glycosylated.

78. The use according to claim 77 wherein the recombinant α -N-acetylglucosaminidase has a molecular weight as determined using SDS/PAGE of
5 at least approximately 79kDa.

79. The use according to claim 78 wherein the recombinant α -N-acetylglucosaminidase has a molecular weight as determined using SDS/PAGE of approximately 79kDa to 89kDa.

10 80. The use according to claim 72 wherein the recombinant α -N-acetylglucosaminidase comprises a sequence of amino acids substantially as set forth in SEQ ID NO:2 or has at least 40% similarity to all or a part thereof.

81. The use according to claim 80 wherein the percentage similarity to SEQ ID NO:2 is at least 60%.

15 82. The use according to claim 80 wherein the percentage similarity to SEQ ID NO:2 is at least 80%.

83. A nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a polypeptide capable of hydrolyzing the terminal α -N-acetylglucosaminidase residues present at the non-reducing terminus of fragments of heparan sulphate and heparin and wherein said
20 nucleotide sequence is capable of hybridising under at least low stringency conditions to the nucleotide sequence set forth in SEQ ID NO:1.

84. A nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding a polypeptide capable of
25 hydrolysing the terminal α -N-acetylglucosaminidase residues present at the non-reducing terminus of fragments of heparan sulphate and heparin and wherein said nucleotide sequence is capable of hybridising under at least low stringency conditions to the nucleotide sequence set forth in SEQ ID NO:3.

85. A recombinant polypeptide comprising a sequence of amino acids corresponding to the amino sequence set forth in SEQ ID NO:2 or having at least 40% similarity thereto and encoded by a nucleic acid molecule which is capable of hybridising to the nucleotide sequence set forth in SEQ ID NO:1 or SEQ ID NO:3 under at least low stringency conditions.

86. A genetic construct comprising the nucleic acid molecule according to claim 1, operably connected in the sense orientation to a promoter sequence such that said genetic construct is capable of being expressed in a eukaryotic or prokaryotic cell to produce a recombinant mammalian α -N-acetylglucosaminidase or a fragment or derivative thereof.

87. The genetic construct according to claim 86 wherein the promoter is capable of regulating expression of the recombinant α -N-acetylglucosaminidase in a mammalian cell.

88. The genetic construct according to claim 87 wherein the promoter is the CMV promoter sequence or a promoter derived therefrom.

89. The genetic construct according to claim 86 further comprising a transcription terminator sequence.

90. The genetic construct according to claim 86 when used to express or over-express α -N-acetylglucosaminidase in a eukaryotic or prokaryotic cell.

91. An antibody to α -N-acetylglucosaminidase or a recombinant α -N-acetylglucosaminidase according to claim 19 or an antigenic fragment thereof.

92. The antibody according to claim 91 further defined as a polyclonal antibody molecule.

93. The antibody according to claim 91 further defined as a monoclonal antibody molecule.

94. The isolated nucleic acid molecule according to claim 8 wherein the percentage similarity is at least 60%.

95. The isolated nucleic acid molecule according to claim 94 wherein the percentage homology is at least 80%.

96. A recombinant mammalian α -N-acetylglucosaminidase or fragment thereof wherein the α -N-acetylglucosaminidase or fragment thereof is in glycosylated form and comprises a sequence of amino acids substantially the same as a human α -N-acetylglucosaminidase.

97. The recombinant mammalian α -N-acetylglucosaminidase according to claim 96 when fused to another proteinaceous molecule.

98. The recombinant mammalian α -N-acetylglucosaminidase according to claim 97 wherein the other proteinaceous molecule is an enzyme, reporter molecule, purification site and/or a signal sequence.

99. The recombinant mammalian α -N-acetylglucosaminidase according to claim 96 comprising an amino acid sequence substantially as set forth in SEQ ID NO:2 or having at least 40% similarity to all or part thereof.

100. The method according to claim 57 wherein administration of the recombinant mammalian α -N-acetylglucosaminidase is by oral, intravenous, suppository, intraperitoneal, intramuscular, intranasal, intradermal or subcutaneous administration by infusion or implantation or by enzyme replacement therapy or by gene therapy.

101. A pharmaceutical composition comprising recombinant mammalian α -N-acetylglucosaminidase or an active fragment or derivative thereof and one or more pharmaceutically acceptable carriers and/or diluents when used in the method according to claim 57.

102. A pharmaceutical composition comprising recombinant mammalian α -N-acetylglucosaminidase or an active fragment or derivative thereof and one or more pharmaceutically acceptable carriers and/or diluents when used in the method according to claim 58.

103. A genetic construct comprising the nucleic acid molecule according to claim 83, operably connected in the sense orientation to a promoter sequence such that said genetic construct is capable of being expressed in a eukaryotic or prokaryotic cell to produce a recombinant mammalian α -N-acetylglucosaminidase or a fragment or derivative thereof.

104. A genetic construct comprising the nucleic acid molecule according to claim 84, operably connected in the sense orientation to a promoter sequence such that said genetic construct is capable of being expressed in a eukaryotic or prokaryotic cell to produce a recombinant mammalian α -N-acetylglucosaminidase or a fragment or derivative thereof.

105. An antibody to α -N-acetylglucosaminidase or a recombinant α -N-acetylglucosaminidase according to claim 28 or an antigenic fragment thereof.

106. The antibody according to claim 15 further defined as a polyclonal antibody molecule.

107. The antibody according to claim 15 further defined as a monoclonal antibody molecule.

108. An antibody to α -N-acetylglucosaminidase or a recombinant α -N-acetylglucosaminidase according to claim 35 or an antigenic fragment thereof.

109. The antibody according to claim 108 further defined as a polyclonal antibody molecule.

110. The antibody according to claim 108 further defined as a monoclonal antibody molecule.